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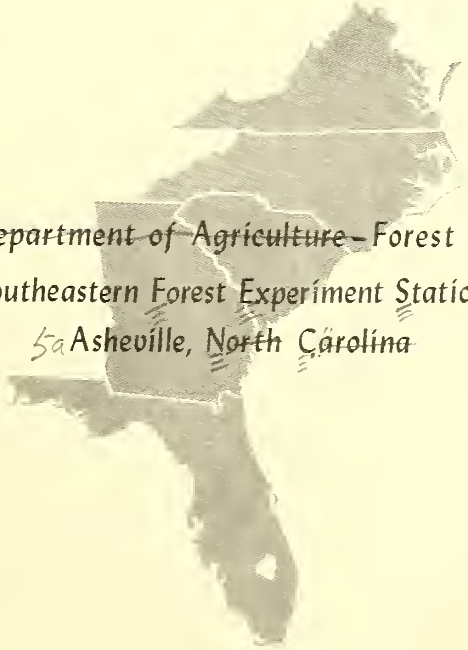
3 The Effect of Hydrogen Peroxide on the Germination of Loblolly and Slash Pine Seed

by

Mason C. Carter and LeRoy Jones



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The Effect of Hydrogen Peroxide
on the
Germination of Loblolly and Slash Pine Seed 1/

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Introduction

Ching and others (2, 3, 5) have reported that dilute solutions of hydrogen peroxide have a pronounced promoting effect upon the germination of the seed of certain western conifers. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was particularly responsive. Seed soaked in 1 percent hydrogen peroxide for 48 hours reached 50 percent germination in $1\frac{1}{2}$ days, whereas seed stratified at 3° C. for 10 days required $8\frac{1}{2}$ days to reach 50 percent germination (2). Stimulations of this magnitude would be quite important in forest nursery and direct seeding operations because a large part of mortality occurs during the germination period (4). Increasing the speed of germination would shorten the time of vulnerability and should reduce mortality.

The current study was carried out to determine the effect of hydrogen peroxide upon the germination of loblolly (*Pinus taeda* L.) and slash (*Pinus elliottii* Engelm.) pine seed.

Experimental Procedure

All studies were carried out at the Eastern Tree Seed Laboratory 2/ located at the Georgia Forestry Center.

1/ This work was conducted while the senior author was employed by the Southeastern Forest Experiment Station, U. S. Forest Service, Macon, Georgia. He is now Assistant Forester, Auburn University, Auburn, Alabama. The junior author is Forester, Eastern Tree Seed Laboratory, U. S. Forest Service, Macon, Georgia.

2/ Operated in cooperation with Region 8 and Southeastern Forest Experiment Station, U. S. Forest Service; Georgia Forestry Commission; and Georgia Forest Research Council.

Each seed test consisted of duplicate samples of 100 seed placed in plastic dishes containing the germination medium. The medium was a 1 to 1, by volume, mixture of vermiculite and washed and sterilized sand. Seed were pressed firmly into contact with the medium and the dishes were watered, covered, and placed in germination rooms where automatic control of temperature and illumination period was maintained (fig. 1).

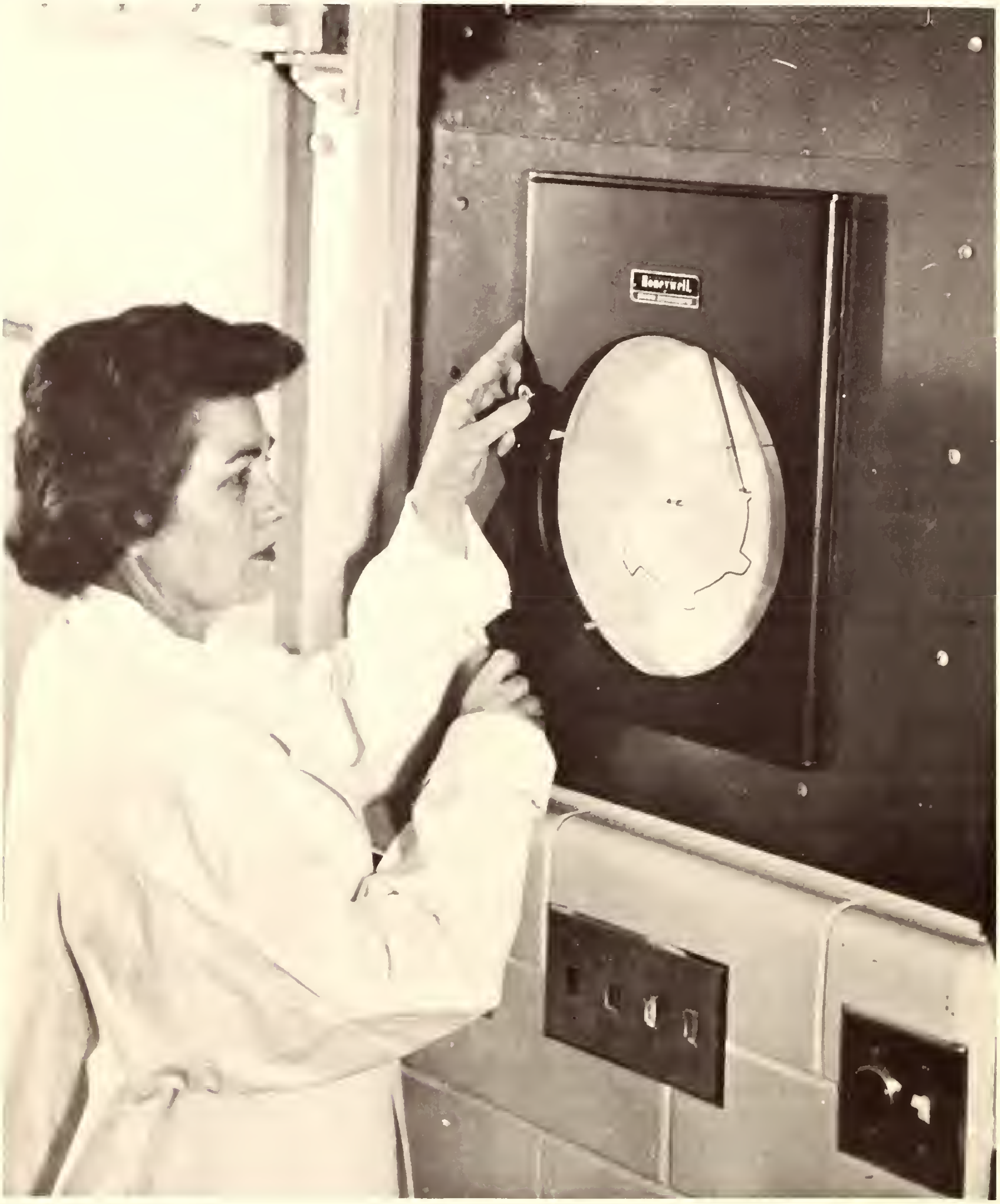

Figure 1.--Temperature and light were controlled automatically. Thermograph shows temperature in the alternating germination room.

Tests were conducted for 30 days. Germination counts were made every 2 days, starting on the third day for slash pine and the sixth day for loblolly pine. A seed was considered normally germinated when the radicle entered the medium and "erected" the seed coat. Abnormal germination was considered ungerminated. At the end of the germination period all ungerminated seed were cracked and germination figures were then expressed in terms of normal percent germination of full seed (fig. 2).

Stratification was accomplished by placing seed in a cloth bag, surrounded by wet peat moss, in a vented plastic container and storing the package at 36° to 38° F. for 30 days.

Experiment 1

Preliminary work indicated that concentrations of hydrogen peroxide greater than 3 percent were injurious to slash and loblolly pine seed. A soaking period of more than 96 hours also appeared to be detrimental.

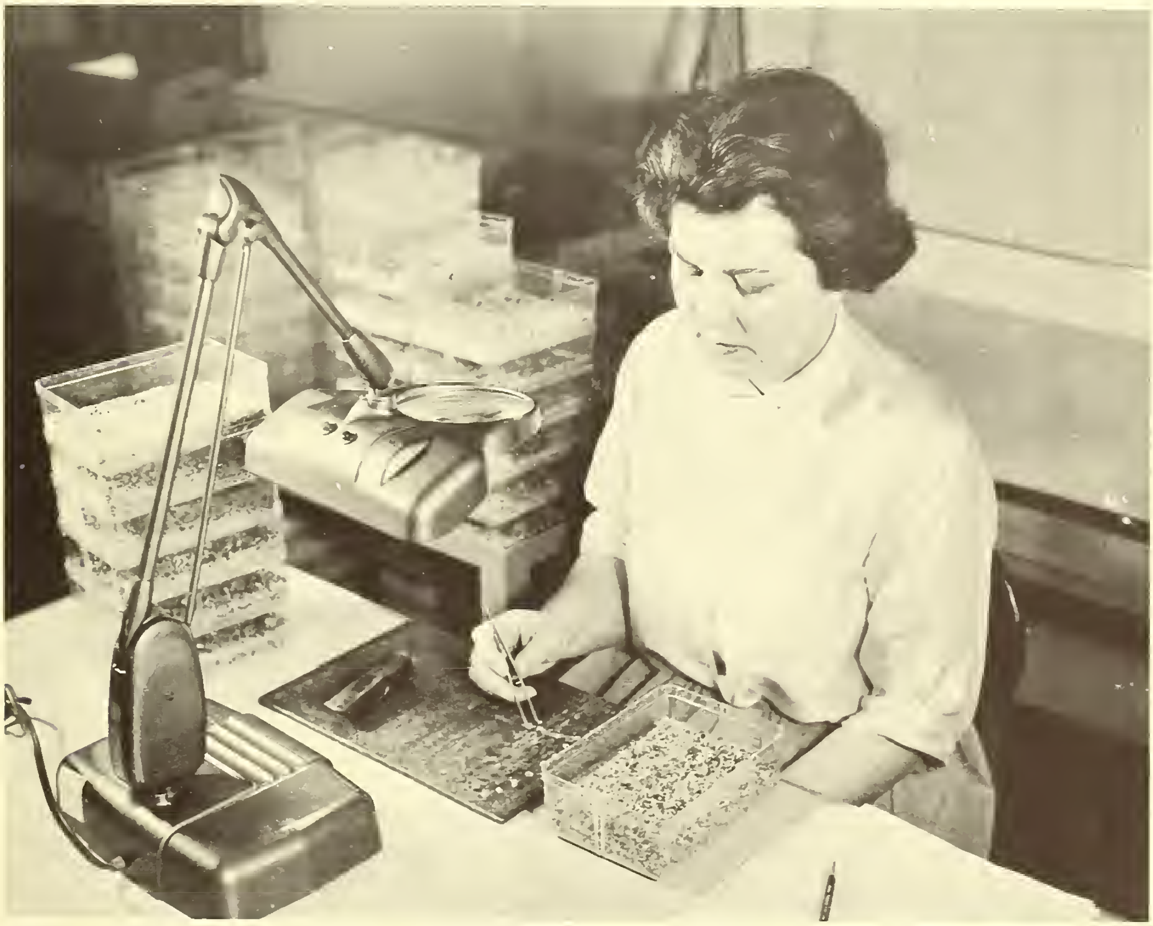

Figure 2.--Ungerminated seed were recovered and cracked so the full seed percent germination values could be used in the analysis. This eliminated any error that could have been attributed to empty seed.

An experiment was designed to compare germination speed and magnitude of seed soaked in hydrogen peroxide, seed soaked in water, and seed stratified with that of untreated or dry seed. The treatments were as follows:

Treatment number	Treatment
1	Untreated seed
2	Stratified seed
3	Seed soaked 24 hours in water
4	Seed soaked 48 hours in water
5	Seed soaked 72 hours in water
6	Seed soaked 96 hours in water
7	Seed soaked 24 hours in 1 percent hydrogen peroxide
8	Seed soaked 48 hours in 1 percent hydrogen peroxide
9	Seed soaked 72 hours in 1 percent hydrogen peroxide
10	Seed soaked 96 hours in 1 percent hydrogen peroxide
11	Seed soaked 24 hours in 3 percent hydrogen peroxide
12	Seed soaked 48 hours in 3 percent hydrogen peroxide
13	Seed soaked 72 hours in 3 percent hydrogen peroxide
14	Seed soaked 96 hours in 3 percent hydrogen peroxide

The germination conditions were also varied. One group of treatments were germinated under alternating temperatures of 86° F. for 8 hours in the light and 68° F. for 16 hours in darkness. Another group was germinated at a constant temperature of 72° F. with 16 hours of light and 8 hours of darkness. The alternating temperature conditions approximated those used by Ching (2); the constant temperature conditions are standard at the Eastern Tree Seed Laboratory (1).

Three different seed lots were used for both slash and loblolly seed. Each lot was divided into two sublots, one for alternating temperature and one for constant temperature. From each sublot, 14 samples of approximately 200 seed were drawn at random (fig. 3). Each sample, except the samples to be stratified, was placed in a 250 ml. Erlenmeyer flask. At daily intervals, 200 ml. of the appropriate solution--water or hydrogen peroxide--were added to the flasks, starting with the 96-hour soakings on the first day, 72-hour soakings on the second day, etc. In this manner, all germination tests were begun on the same day. The stratified samples were drawn 30 days prior to the start of the germination test so that germination of this test was begun on the same day as the other tests.

Experiment 2

A second experiment was designed to study the effect of volume of peroxide solution, storage after treatment, and frequent changing of solution upon the germination rate of loblolly pine seed. A 1 percent solution of hydrogen peroxide was used for all of the treatments. There were 3 soaking periods--24, 48, and 72 hours; two volumes of solution--25 ml. and 100 ml. per 100 seed; solutions were changed daily in half the treatments and unchanged in the other half; samples from each treatment were set up for germination immediately or surface-dried and stored 3 days at 38° F. All treatments were duplicated and germinated at a constant temperature of 72° F. with 16 hours of daylight and 8 hours of darkness per day.

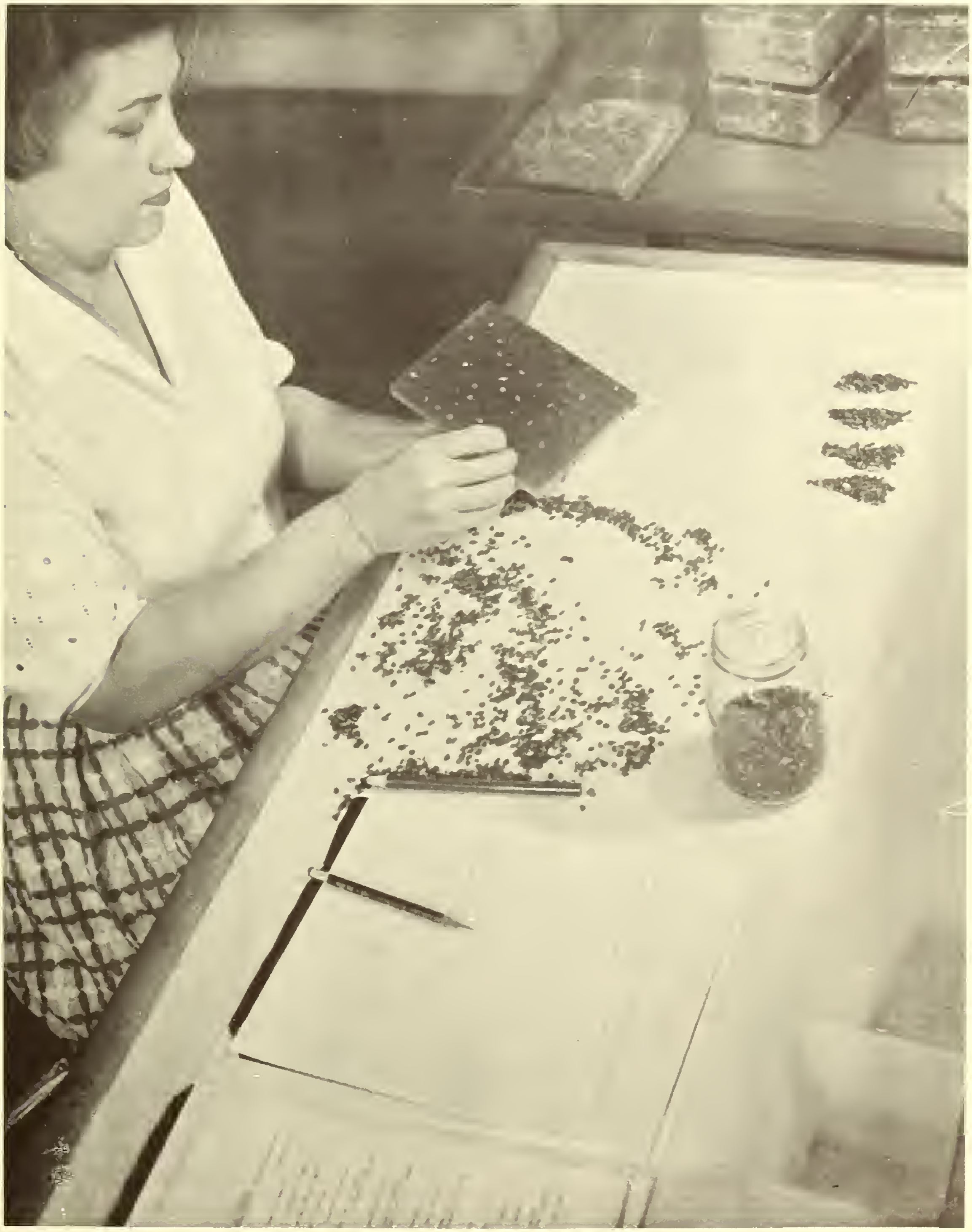

Figure 3.--Seed were randomly selected with a vacuum seed counter.

Results

Experiment 1

With loblolly pine, the germination data for the 10th and 30th days after the start of the germination tests were analyzed. For slash pine, the data from the 7th and 30th days were used. The 7th and the 10th days after the start of the tests represent the points where approximately 50 percent of the total germination had occurred for slash pine and loblolly pine, respectively. The germination of both species was essentially complete by the 30th day. By using the two sets of figures, both the speed and extent of germination could be studied.

The full-seed germination percentage was calculated for each observation and the percentages were transformed by arcsin$\sqrt{\text{percent}}$ (6). An analysis of variance was then performed upon the transformed values (table 1).

The germination rates for loblolly seed were not affected by germination conditions; however, the germination rates for slash seed were significantly lower under alternating temperature conditions than they were under constant temperature conditions (tables 1 and 2).

Differences between treatments were highly significant at both times of observation for each species. Significance at the 1 percent level was apparent between the three soaking solutions (water, 1 percent hydrogen peroxide, and 3 percent hydrogen peroxide) at all times of observation.

Single degree of freedom breakdown of the effects of solutions indicated that hydrogen peroxide treatment was vastly superior to water. The 1 percent solution of hydrogen peroxide was superior to the 3 percent solution in every instance except the final readings for slash seed.

The linear component and the main effect of soaking periods were highly significant in the slash pine data but unsignificant in the loblolly. Most of this effect may be because increased soaking time in water sharply decreased the germination of slash seed. The interaction between soaking period and solution was also highly significant with the most significant component being the interaction of the linear component of soaking period with the water versus hydrogen peroxide component of the solution effect.

The average of stratified plus unstratified treatments was significantly less than the average of all other treatments for the 7th- and 10th-day observations for slash and loblolly, respectively. These effects were not significantly different at the time of the final germination counts. The soaking treatments significantly increased the speed of germination over the average of stratified plus unstratified but did not increase the total germination.

Table 1.--Analyses of variance for experiment 1

Source	Degrees of freedom	Mean squares[1]			
		Loblolly		Slash	
		10th day	Final	7th day	Final
Reps	2	102.21	326.62	4391.08**	6547.04**
Germination conditions	1	163.85	747.66	491.76*	313.35*
Error "a"	2	320.78	87.66	8.16	16.15
Treatments	13	328.61**	88.80**	390.66**	358.24**
Between solutions (S) (H_2O, 1 percent H_2O_2, 3 percent H_2O_2)	2	1083.41**	306.80**	1546.46**	1195.18**
(1) H_2O vs. average of H_2O_2	1	1761.95**	263.78**	2891.74**	2348.69**
(2) 1 percent H_2O_2 vs. 3 percent H_2O_2	1	404.88**	349.81**	201.17*	41.67
Soaking period (T)	3	140.22	58.79	261.26**	407.49**
(a) Linear	1			689.18**	1128.58**
(b) Quadratic	1			67.13	4.92
(c) Cubic	1			27.47	88.98
S X T	6	32.47	51.36	175.94**	105.06**
(1) x (a)	1			826.66**	451.66**
(1) x (b)	1			6.11	5.52
(1) x (c)	1			13.31	8.86
(2) x (a)	1			49.26	87.80
(2) x (b)	1			160.19*	75.95
(2) x (c)	1			0.10	0.75
Unstratified and stratified vs. other treatments	1	251.34*	0.42	126.07*	6.90
Unstratified vs. stratified	1	1238.26**	55.81	20.22	406.97**
Conditions x treatments	13	41.59	16.76	70.54*	74.16*
Error "b"	52	57.74	32.83	30.11	33.11
Sampling error	84	8.16	10.46	10.75	14.78

[1] Single degree of freedom breakdowns not shown for nonsignificant main effects.

* Indicates significance at the 5 percent level.
** Indicates significance at the 1 percent level.

Table 2.--Germination means under the different germination conditions

Germination conditions	Loblolly		Slash	
	10th day	Final	7th day	Final
Constant temperature 72° F. - 16 hrs. light	54.4	82.9	64.7	76.9
Alternating temperature 86° F. - 8 hrs. light 68° F. - 16 hrs. dark	57.8	77.1	59.2	72.5

Stratified loblolly seed exhibited a significantly higher germination than unstratified on the 10th day of germination but not on the final day, indicating a typical response to stratification. Stratification had no effect upon the germination of slash seed on the 7th day of germination but produced a significant reduction in the total germination (tables 1 and 3). It is not uncommon for stratification to have a detrimental effect upon germination of slash seed (1).

There were no significant interactions between germination conditions and treatments in the loblolly data. However, this interaction was significant in the slash data. One of the slash pine seed lots was a particularly poor lot having an average germination of 56 percent, compared to 82 percent and 85 percent for the other two lots. The erratic response of this one poor lot resulted in a highly significant difference between lots and greatly contributed to the significant interaction between treatments and germination conditions. Stratified seed from the poor lot germinated better under alternating temperatures than under constant temperature conditions, whereas with peroxide-treated seed the reverse was true. No other trends were obvious, which might explain the significance of the interaction.

Table 3.--The treatment means averaged over replications and germination conditions for experiment 1

Treatment	Loblolly		Slash	
	10th day	Final	7th day	Final
Unstratified	[1] 38.9	82.6	57.1	80.8
Stratified	62.7	77.6	59.8	68.9
Water - 24 hours	45.9	78.9	64.6	78.0
Water - 48 hours	48.0	79.0	55.0	67.5
Water - 72 hours	50.5	78.4	50.6	64.6
Water - 96 hours	50.0	72.9	38.7	53.8
Average for water	48.6	77.3	52.2	66.0
1 percent hydrogen peroxide - 24 hours	56.2	81.9	69.4	83.0
1 percent hydrogen peroxide - 48 hours	66.1	84.3	73.2	81.3
1 percent hydrogen peroxide - 72 hours	65.6	83.9	73.1	80.9
1 percent hydrogen peroxide - 96 hours	70.1	85.8	63.9	73.5
Average for 1 percent	64.5	84.0	69.9	79.7
3 percent hydrogen peroxide - 24 hours	54.2	82.7	65.9	80.5
3 percent hydrogen peroxide - 48 hours	59.0	81.4	64.3	76.7
3 percent hydrogen peroxide - 72 hours	61.2	76.2	65.1	78.6
3 percent hydrogen peroxide - 96 hours	56.8	74.8	66.6	77.2
Average for 3 percent	57.8	78.8	65.5	78.3

[1] All figures are percent germination of full seed.

Experiment 2

An analysis of variance was performed upon the full seed germination percent values after transformation to arcsin. Germination percentages for the 9th and final (30th) days of the tests were used.

The only significant comparison on the 9th day of germination was the difference between seed germinated immediately after treatment and seed stored 3 days before the start of germination (tables 4 and 5). When germination was complete, the effect of storage was no longer apparent, indicating that storage for 3 days destroyed the stimulating effect of hydrogen peroxide upon the speed of germination but did not reduce the total germination.

The interaction between length of the soaking period and volume of peroxide solution was significant on the final day of germination. With 48 or 72 hours of soaking, the lower volume (25 ml./100 seed) was equal to the higher volume (100 ml./100 seed), but with a 96-hour soak, there was a depressing effect of the lower volume (table 6).

Discussion and Conclusions

The results reported here are in general agreement with those reported previously for other species (2, 3, 5) and indicate that soaking in 1 percent hydrogen peroxide solution may be a suitable substitute for stratification to increase the speed and uniformity of germination of loblolly and slash pine seed. In some instances the total germination of loblolly pine may be increased by treatment with hydrogen peroxide.

For loblolly pine seed, a 48- to 96-hour soak in 1 percent hydrogen peroxide was the most successful treatment. The 24-hour soak was definitely inferior to the longer treatments. With slash pine seed, however, the 24-hour treatment was equal, or superior, to the longer exposures to 1 percent H_2O_2. The difference in response between the two species probably is caused, in part, by differences in the thickness of the seed coats of the two species. Loblolly pine possesses a much heavier seed coat than slash pine and the peroxide may not penetrate to the interior of loblolly pine seed as rapidly as it does in slash pine.

The 1 percent solution of hydrogen peroxide was superior to the 3 percent solution in nearly every instance. The longer soakings in 3 percent solution appeared more damaging to loblolly pine seed germination than to slash pine seed. Preliminary studies indicated that soaking in 5 percent and 10 percent hydrogen peroxide solutions for just a few hours may be lethal to both species, and prolonged soakings in 3 percent solution may drastically reduce germination.

Table 4.--Analysis of variance for experiment 2

Source	Degree of freedom	Mean square	
		9th day[1]	Final[2]
Solution change vs. solutions not changed (C)	1	55.06	0.05
Soaking period (D)	2	40.87	14.98
Storage (S)	1	[3]712.17	7.93
Volume (V)	1	16.98	19.88
C x D	2	62.37	7.20
C x S	1	48.43	24.29
C x V	1	0.25	5.67
D x S	2	50.52	5.33
D x V	2	2.60	[4]32.26
S x V	1	2.04	3.68
Error: C x D x S C x D x V C x S x V D x S x V C x D x S x V	9	32.48	7.06 [5]6.83
Sampling error	24	7.12	6.74
Total	47		

[1] All M. S. tested against pooled value of 3rd and 4th order interactions termed "Error."
[2] All M. S. tested against pooled value of "Error" plus Sampling error.
[3] Significant at 1 percent level.
[4] Significant at 5 percent level.
[5] Pooled error term.

Table 5.--The effect of storage after treatment upon the germination of seed soaked in 1 percent hydrogen peroxide

Treatment	Percent germination
No storage	[1]52.5
Stored 3 days at 38° F.	39.3

[1] Figures represent germination on the 9th day and were significantly different at the 1 percent level.

Table 6.--The effect of length of soaking and volume of solution of 1 percent hydrogen peroxide upon the germination of loblolly pine seed

Volume per 100 seed	Soaking period		
	48 hours	72 hours	96 hours
25 ml.	[1] 85.8	85.1	80.1
100 ml.	86.4	83.5	86.6

[1] Figures represent mean germination percentage values on the final (30th) day of germination. The interaction of the two factors was significant at the 5 percent level.

A daily change of the peroxide solution and different volumes of solution had no significant effect upon the germination of loblolly pine seed. There was a significant interaction, however, between the length of the treatment period and solution volume, indicating that the use of the higher volume yielded higher germination rates when the seed were subjected to prolonged soaking.

The detrimental effect of storage following hydrogen peroxide treatment was not surprising, but the magnitude of the effect was greater than had been anticipated. Evidently the stimulating effect of hydrogen peroxide is short lived and is lost in a very short time in cold storage.

It appears that for a nursery or direct seeding operation, a 1-day soak for slash and a 2- or 3-day soak for loblolly pine in 1 percent hydrogen peroxide should be best. Between 4 and 5 gallons of solution would be needed per pound of seed. The seed would have to be sowed within a few hours after removal from the hydrogen peroxide solution, and it might be preferable to leave the seed in peroxide for an additional 24 hours if conditions were not suitable for sowing at the end of the original treatment period.

Current studies are attempting to develop a practical procedure for the use of hydrogen peroxide treatment in a nursery operation. One of the factors difficult to evaluate is the cost of hydrogen peroxide treatment. If purchased in bulk quantities, technical grade 30 percent hydrogen peroxide costs approximately 20 cents per pound. At this rate, treatment with hydrogen peroxide would cost around 5 cents per pound of seed for peroxide alone. The cost of labor cannot be estimated until a suitable nursery procedure is developed. It appears that the cost of hydrogen peroxide treatment would not be prohibitive.

Summary

The effect of hydrogen peroxide upon the germination of slash and loblolly pine seed was studied. A 48- to 96-hour soak in 1 percent hydrogen peroxide increased the speed of germination of loblolly pine seed to the point where it equalled that obtained by stratification. However, this treatment had no effect upon the total germination. Soaking the slash pine seed in 1 percent hydrogen peroxide for 24 to 48 hours increased both the rate and extent of germination beyond that of stratified seed. Changing the peroxide solutions daily and varying the solution volume did not alter the stimulating effect of the H_2O_2, but storage of the seed at 38° F. for 3 days following treatment produced a significant reduction in the germination rate.

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